PCR Master Mix with Dye (2X)

Catalog: NP100056

Size: 5 mL

Concentration: 2X

Components:

PCR Master Mix with Dye (2X)

Product Description

OriGene's PCR Master Mix (2X) is an optimized premix containing DNA polymerase, dNTPs, $MgCl_2$, KCl and other stabilizers. The template can be purified DNA, bacterial colonies/bacteria liquid, crude extract or cDNA, etc. This product can use complex genomic DNA as a template to amplify a target fragment of 5 kb in length or a simple template such as lambda DNA to amplify a target fragment of 10 kb in length. It is suitable for applications such as PCR reaction, colony PCR identification, vector construction and so on. etc.

Storage: -20℃

Product Source:

The DNA polymerase gene was induced and expressed in *E. coli* and obtained by separation and purification

Thermal Inactivation: No

5´-3´exonuclease activity: No

3´-5´exonuclease activity: Yes

Fidelity : 6X *Taq*

Product End: Blunt end

Operation Description

Standard Protocol:

- It is recommended to prepare all reaction components on ice, and then quickly transfer the reaction system to a thermocycler preheated to 98℃ .

Recommended Reaction:

Components	25 μL	50 μL	Total Concentration
PCR Master Mix (2X)	12.5μL	25 μL	1X
Forward Primer (10 μM)	0.5 μL	1 μL	0.2 μM
Reverse Primer (10 μM)	0.5 μL	1 μL	0.2 μM
DNA Template*	Variable	Variable	<300 ng
Nuclease-free Water	to 25 μL	to 50 μL	N/A

** Note: The optimal reaction concentration varies with different DNA templates. Please refer to the basic principles of PCR below.*

Recommended PCR Program:

Step	Temp	Time	Cycles
Pre-denaturation	98℃	45 s	1
Denaturation	98℃	10 s	30
Annealing	55-65℃	30 s	
Extension	72℃	20-30s/kb	
Post-extension	72℃	5min	1
Hold	4-12℃	∞	1

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PCR Principles:

1. Template:

High-quality purified DNA templates are important to high-fidelity PCR reactions. The recommended DNA template amounts with different complexity are listed Below (For a 50μL reaction):

DNA	Input Amount
Plants, animals and human gDNA	10 ng~100 ng
*E.*coli，lambda gDNA	500 pg-200 ng
Plasmid DNA	1 pg~10 ng

Note: If the DNA template is obtained from a cDNA synthesis reaction, the template volume should be less than 10% of the total reaction volume. If long fragments are amplified, the amount of template input should be increased appropriately

2. Primers:

Oligonucleotide primers are typically 20-40 nucleotides in length with a GC content of 40-60%. Primers can be designed and analyzed using software such as Primer 3. The final concentration of each primer in the PCR reaction system should be in the range of 0.1-1 μM.

3. Denaturation:

98℃ pre-denaturation for 45 s can fully denature most DNA templates. In the case of high complexity DNA templates, the pre-denaturation time should be extended up to 3 minutes for fully denaturation. Generally, the recommended denaturation condition for low-complexity DNA templates is 98℃, 5-10 s

4. Annealing:

The annealing temperature of PowerPol 2X PCR Mix is usually higher than other PCR polymerases. Generally, primers longer than 20 nt are annealed at (lower primer Tm+3)℃ for 10-30 s; when the primers are shorter than 20 nt, an annealing temperature equivalent to the lower primer Tm. When using a new primer set for PCR reaction, we recommend a gradient PCR to determine the optimal annealing temperature. In a two-step amplification protocol, the annealing temperature should be set to the extension temperature.

5. Extention:

The recommended extension temperature is 72℃. The extension time depends on the length and complexity of the amplicon. For the low-complexity amplicons (plasmid DNA), the extension condition is 10 s/kb. For high-complexity amplicons, it is recommended to increase the extension time to 20-30 s/kb. In some cases, the extension time for cDNA templates should be less than 1 min/kb

6. Cycles:

To obtain enough yield of PCR products, 25-35 cycles are recommended.